Review

Omega-3 PUFA metabolism and brain modifications during aging

Laurie Chevalier*, Hillary Chappus-McCendie*, Claude Roberge and Mélanie Plourde[1,#]

[1] Research Center on Aging, Health and Social Services Centre – University Institute of Geriatrics of Sherbrooke, Department of medicine, Université de Sherbrooke, 1036 Belvédère Sud, Sherbrooke, Canada, J1H 4C4; E-Mails: Melanie.plourde2@usherbrooke.ca (M.P);

*LC and HCM equally contributed to the redaction and the revision of the manuscript and are considered co-first authors.

#Author to whom correspondence should be addressed;

E-Mail: Melanie.plourde2@usherbrooke.ca

Phone number: +1-819-780-2220 extension 45664;

Fax: +1-819-829-7141.

Abstract:

In Canada, 5.5 million (16% of Canadians) adults are >65 years old and projections suggest this number will be approximately 20% of Canadians by 2024. A major concern regarding old age is a decline in health, especially if this entails a loss of self-sufficiency and independence caused by a decline in cognition. The brain contains 60% of fat and is one of the most concentrated organs in long chain omega-3 fatty acids such as docosahexaenoic acid (DHA). During aging, there are physiological modifications in the metabolism of lipids that could also have consequences on brain structure and levels of DHA. This review will hence discuss the physiological modifications in the metabolism of lipids during aging with a focus on long chain omega-3 and omega-6 fatty acids and also outline the structural and functional modifications of the brain during aging including brain lipid modifications and its relation to higher levels of DHA and cognition. Therefore, in this review, we outline the importance of collecting more data on the biology of aging since it might highly improve our understanding about what are «normal» modifications occurring during aging and what can become pathological.

Keywords: lipid metabolism, aging, docosahexaenoic acid, fatty acids, brain structure, brain function,

1. Introduction

Almost every country in the world experiences an aging population, and this population is expected to be one of the most significant forces shaping our economy and society in the next 20-30 years. A major concern about old age, both at the individual and societal levels, is a decline in health, especially if this means a loss of self-sufficiency and independence. Increasing research aimed at promoting healthy aging is actually ongoing but one of the major hurdles is to define the biology of aging. Aging in humans refers to a multidimensional process of physical, psychological, and social changes. Therefore, it follows that fundamental knowledge on the biological processes occurring during aging may help to design environmental strategies aimed at promoting healthy biological aging. Thus, there is a need for better prevention strategies, but one major gap in this field is a need to better understand what the biological modifications are, also called geroscience, since this field is relatively new. One of the strategies to promote healthy aging is the consumption of one or two fish meals each week [1-3]. Normally, the intake of fish positively correlates with increased plasma and erythrocyte omega-3 fatty acids (n-3 FA), likely with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations in a time- and dose-dependent manner [4-6]. EPA and DHA have to be provided through the diet because their synthesis from their precursor alpha-linolenic acid (ALA) is extremely limited in humans [7]. However, over the 20th century, the dietary fat consumption has drastically changed with an increased level of omega-6 fatty acids such as linoleic acid (LA) from 2.79% to 7.21% of energy. This shift in our dietary fat intake was largely due to our dependence on new food production methodologies, including soybean oil [8].

The link between our dietary fat intake and the incidence of chronic diseases has been largely debated over the last 20 years. Our research group is mainly focused on prevention of cognitive decline, so the focus of this review paper, with respect to chronic diseases, will be on cognition. This link between dietary fat intake and the risk of cognitive decline has been the focus of many review papers overs the last 10-15 years [9-11]. One of the most recent reviews supports a positive association between dietary and blood n-6: n-3 ratio and cognitive decline and incidence of dementia, as evaluated on 14 human studies including 7 prospective studies [12]. A recent meta-analysis on 11 cohort studies evaluated the association between 299 metabolites and general cognitive ability and dementia. They reported that higher DHA levels in blood were associated with higher cognitive function in 22,887 individuals [13]. Hence, it seems that more elevated concentration of n-3 FA in the blood is associated with lower cognitive decline and perhaps lower risk of other chronic diseases. However, our group showed that for older participants, plasma EPA and DHA kinetics are dysregulated and this will likely lower the capacity of older adults to incorporate EPA and DHA in organs

and tissues. Usually, a fish oil supplementation increases the level of EPA and DHA in the plasma or erythrocytes but in those aged >70 years old, we don't know whether this process is efficient. There is no clear definition or parameters to define an old *vs.* a young participant. Most of the studies used the median of age in their participants group or a continuous age ae range. Following from the information summarized above, this paper will review some of the metabolism modifications occurring during aging with a focus on lipid metabolism. By reviewing these evidences, we will also expose how these modifications might limit incorporations of n-3 FA in membranes of cells with a focus on the brain because it is one of the most enriched organs in DHA.

2. Lipid and fatty acid metabolism differences during aging

Generally speaking, there are differences in the lipid and fatty acid metabolism occurring during aging and these modifications are considered totally normal and part of the aging process. These processes include the transport of fatty acids after their intake and their transit to the different organs and tissues that are modified during aging. This section will review some of these modifications.

2.1.Normal transport of fatty acids from dietary intake to their circulation in the blood:

In Western adults, the diet is composed of 30 to 40% of lipids, of which 92 to 96% are long chain fatty acid esterified to a glycerol thus constituting what is the main form of dietary lipid: triglycerides (TG) [14]. Whole-body homeostasis requires fine-tuning of fatty acid transport and utilization by metabolically active tissues [15]. Because of their regulatory roles in cellular fatty acid uptake and utilization, membrane apolipoprotein receptors and fatty acid transporters form an integral part of this homeostatic system. As a result, imbalances in lipid metabolism likely will influence the functioning of fatty acid transporters and their protein levels. Lipids are not soluble in water and necessitate incorporation into amphiphilic molecules called lipoproteins to circulate in the blood. Hence, following ingestion of TG, they will be hydrolysed at their ester bonds by gastric and pancreatic lipases into two non-esterified fatty acids (NEFA) and one monoacylglycerol (MAG) with the fatty acid being in the *Sn*-2 position [16]. Both forms of lipids are passively transported into enterocytes [17] via diffusion or transporters such as "Fatty Acid Transport Proteins" (FATPs) and "Cluster of Differentiation 36" (CD36) [18]. Dietary lipids are efficiently digested and absorbed by the enterocytes [19].

Once inside the intestinal cells, enzymes convert the NEFAs and MAG back into TG [20]. These will be integrated in chylomicrons and exported to the lymphatic system via the Golgi apparatus [21].

The chylomicrons, now rich in exogenous triglycerides, join the bloodstream via the thoracic duct and get transported to the peripheral tissues such as muscle and fat cells. In the bloodstream, lipoprotein lipase (LPL) gets activated when it detects an apolipoprotein C II (apoC-II) [22] on the surface of the chylomicrons. The role of lipoprotein lipase is to hydrolyse the ester bonds of TGs in chylomicrons [22] to release NEFAs into the bloodstream where there will be an uptake by nearby cells. The loss of TGs will result in a decrease in size of chylomicrons and leave chylomicrons constituents available for the synthesis of native HDL disks [23]. Remnant chylomicrons rich in cholesteryl esters will be captured by endocytosis by hepatocytes receptors such as LDL receptor (LDLR) [22] and LDL receptor-related protein (LRP) (https://onlinelibrary-wiley-com.ezproxy.usherbrooke.ca/doi/abs/10.1002/%28SICI%291096-9136%28199708%2914%3A3%2B%3CS75%3A%3AAID-DIA449%3E3.0.CO%3B2-9). The liver can then use the endogenous TG and cholesteryl esters to form the very low density lipoprotein (VLDL) [24]. These lipoproteins will be directed to peripheral tissues. Following a loss of TG, there will be a decrease in VLDL density [25]. With the action of lipoprotein lipase, VLDL will then become intermediate density lipoprotein (IDL). With the action of hepatic lipase [25] IDL becomes low density lipoprotein (LDL). LDLs carry cholesterol to tissues [26]. LDL will be captured by their receptor, LDLR which are found on cell membranes, where it will be eliminated from the bloodstream by endocytosis [26]. LDL cholesterol will be recovered in the cell. An excess of cholesterol in the tissues will cause an inhibition of transcription of the genes responsible for the formation of the LDLR [27]. It thus reduces the uptake of LDL by the cells and these LDLs will remain in circulation. The remaining LDLs in the circulation are more likely to be oxidized [28] which will thereafter contribute to the development of atherosclerotic plaque [28].

2.2 Lipoprotein metabolism modification during aging

During aging, the metabolism of lipids is modified and causes an increase of plasma lipids. For instance, the fasting plasma levels of VLDL, TG, LDL and cholesterol [29] are significantly higher in the elderly [30]. Higher levels of lipid and cholesterol can be the source of many health problems such as cardiovascular disease and diabetes (REF =http://diabetes.diabetesjournals.org/content/46/8/1354.full-text.pdf + https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4587882/).

These plasma lipid changes in the elderly can cause an increase in plasma free fatty acid levels (https://eds-b-ebscohost-com.ezproxy.usherbrooke.ca/eds/pdfviewer/pdfviewer?vid=1&sid=3f462f39-8acd-4cbf-

b113-b378e62a44c1%40sessionmgr103). Increasing plasma FFA may result in increased plasma glucose by decreasing glucose uptake into the cells. The enzymes responsible for the oxidative cascade of GLA are intimately related to that of glycolysis. Thus, increased lipid oxidation inhibits glucose metabolism, decreases glucose uptake in cells, and impairs glycogen storage http://diabetes.diabetesjournals.org/content/diabetes/37/6/667.full.pdf.. This promotes hyperinsulinemia and ultimate insulin resistance https://eds-b-ebscohost-com.ezproxy.usherbrooke.ca/eds/pdfviewer/pdfviewer?vid=1&sid=3f462f39-8acd-4cbf-b113-b378e62a44c1%40sessionmgr103.

Insulin resistance, often seen in the elderly (https://www.jci.org/articles/view/110908/pdf), will also cause an increase in VLDL and blood triglycerides. Insulin resistance impairs the metabolism of chylomicrons, VLDL, LDL and HDL [31] since a lack of insulin or a lower sensitivity to insulin will reduce the catabolism of chylomicrons and VLDL by LPL. During aging, there is also a higher level of LDL which remains transient for a longer period of time in the plasma (Einarsson K, Nilsell K, Leijd B, Angelin B. Influence of age on secretion of cholesterol and synthesis of bile acids by the liver. N Engl J Med 1985;313(5):277-82. doi: 10.1056/NEJM198508013130501.) as a reference of this statement. = REF 30). In the long term, these LDLs are more likely to be oxidized [31]. The higher concentration of VLDL and chylomicrons in addition to oxidized LDL accumulation in older insulin-resistant individuals would increase the risk of developing cardiovascular disease (CVD) [32]. Furthermore, the increase of LDL may be due to the diminution of bile synthesis from cholesterol by the liver during aging [30, 33]. The decrease in bile acid synthesis is due to the decrease in the expression of "cholesterol 7-alpha hydroxylase" (CYP7A1) during aging. This cytochrome is one of the CYP450 and regulates the formation of bile acids [34]. This causes a decrease in the use of cholesterol by the liver as well as a reduction in LDLR expression with age. Thus, plasma LDL will have lower clearance with age resulting in an increase in plasma LDL concentration in the elderly [29]. In the end, it is possible that deregulation of LDL in the elderly is due to several different phenomena stemming from the large amount of change that occurs with age. The decrease in LDL in the elderly has shown a reduction in the incidence of CVD [35]. In particular, a study showed that long chain polyunsaturated fatty acids (PUFAs) allowed an increase in LDLR expression [36], which could increase the clearance rate of plasma LDL in the elderly and reduce the incidence of CVD. These are some of the modification of the lipid metabolism occurring during aging. Overall, there are usually higher TG and LDL levels in the blood of older adults and it is important to consider these modifications in the prevention of chronic diseases but also when interpreting results pertaining to fatty acid metabolism.

2.3 Omega-3 fatty acid metabolism during aging

Over the last 10 years, our group has worked on omega-3 metabolism with a focus on modifications that occur during aging. This section will report the evidence of omega-3 fatty acid metabolism in three different conditions: before supplementation with omega-3, during or after supplementation with omega-3 fatty acid, and kinetics studies using uniformly labeled carbon 13 fatty acids (^{13}C-).

2.3.1 Without an omega-3 fatty acid supplementation

To our knowledge, there are ~ 24 studies that have reported the level of omega-3 fatty acids or the omega-3 index in young versus old adults (Table 1). Most of the studies reported the fatty acid profile in red blood cells or in plasma/serum phospholipids (PL). Among the 24 studies, 7 studies reported the omega-3 index only and showed that it was higher in older participants [37-43]. Two studies on the omega-3 index reported an increase of about 5-7% of the omega-3 index every decade [37, 41]. Eleven studies reported the fatty acid profile in red blood cells (RBC) [37, 38, 41-50]. For most of the studies, it is difficult to compare the results since the data were not expressed the same way. For instance, two studies reported that the participants having the highest level of omega-3 were on average 8-10 years older than those with the lowest omega-3 fatty acid levels in erythrocytes [43, 44]. Other studies reported the level of increase in omega-3 fatty acids for each increasing decade. Hence, it is difficult to draw a clear conclusion for the omega-3 fatty acid results in RBC but it appears that at older ages, there is more omega-3 in RBC. It is important to note that these papers did not include a complete fatty acid profile of the RBC as it was recently recommended in a paper describing the best practices for the design, laboratory analysis and reporting of clinical trials involving fatty acids [51], hence limiting comparisons between studies. With respect to plasma/serum PL, there were eight studies [45, 52-58]. Six of these studies reported on average a 1.5 fold higher level of DHA in the plasma PL of older participants, aged between 50-88 years old compared with younger participants, aged between 20-49 years old [45, 53-57]. One study reported a 2 fold higher level of EPA in plasma PL but there were no difference between ages for DHA [52]. Yet another study reported only a positive correlation between age and EPA+DHA in plasma PL but it was not possible to quantify the magnitude of the difference between young and older adults [58]. Overall, there is generally good evidence supporting the idea that during aging, the relative % of omega-3 fatty acids or its concentration in RBC and plasma/serum are

higher in the oldest participants compared to the youngest. Some of the proposed mechanism includes a reduction of omega-3 fatty acids in cell membranes, higher intestinal absorption during aging, higher availability and release of adipose tissue stocks. Hence, the exact mechanism behind this higher level of blood omega-3 in older individuals might be multi-level but the important point here is that they might be associated to longevity.

2.3.2 With an omega-3 fatty acid supplementation

To our knowledge, there are nine published studies specifically addressing EPA and DHA responses to an omega-3 fatty acid supplement with participants of different ages (Table 2). Supplementation doses range from 300 mg/d to more than 4 g/d and lasted between 6 weeks and twelve months. Seven studies evaluated the fatty acid profile in the plasma whereas one study evaluated the fatty acid profile in erythrocytes only [59] and another did so in platelets and adipose tissues only [60]. One study reported the omega-3 index pre- and post-supplementation [59] and showed that a low omega-3 index at baseline and an older age predicted those with a greater increase of the omega-3 index after supplementation [59]. This study had similar results to Vandal et al., [61] which showed that the oldest had a higher increase in DHA compared to the youngest after the supplementation, but in their study, Vandal had similar DHA levels in young and old participants at baseline.

The other studies investigated the plasma level of omega-3 fatty acids. One study reported that older participants had higher omega-3 levels at baseline but after the supplementation, the increase was similar in both groups [62]. The six other studies reported a higher increase in EPA [63-67] and/or DHA [68] in older participants compared to younger. The exact mechanism explaining this effect is unclear. Most of the studies reported that it is unlikely that the age-related differences in EPA and DHA at baseline are due to differences in intake of omega-3 PUFA with age. Rather it seems to be related to age differences in endogenous production and incorporation of EPA and DHA due to hormones and hormone sensitivity, body composition, and physical activity, all of which change with age [67]. The study of Walker et al. also showed that the adipose tissue stores less DHA with age in response to EPA + DHA supplementation, hence suggesting that age-related differences in the handling and storage of exogenous supplied DHA may be related to impaired insulin sensitivity with aging or to differences in body composition with aging [67]. The adipose tissue represents a significant store of EPA and DHA, containing the equivalent of several hundred days of the fatty acid content of a typical diet. Altogether, these results support that providing a supplement of omega-3 fatty acid to older adults increases their blood levels when compared to younger individuals. These results may be caused by the fact that older individuals have shown to be more compliant to treatments

than younger people (REF = https://onlinelibrary.wiley.com/doi/full/10.1046/j.1365-2710.2000.00315.x), causing a higher level of omega-3 in their blood. But despite that fact, those results brings into question whether this type of supplementation is useful to them in the prevention of chronic diseases since they may not be able to use it. Another important point is that it might also be due to their lower turnover of circulating TG, hence contributing to their higher omega-3 levels, since omega-3 fatty acid levels are esterified in TG. To answer some of these questions, employing ^{13}C-fatty acids is useful.

2.3.3 Using ^{13}C-fatty acid to evaluate their kinetics during aging

Tracing metabolism of ^{13}C-fatty acids may provide some insight into possible age-related changes in fatty acid metabolism in humans. Metabolism of ^{13}C-DHA has been investigated in humans [69-71]. In young adults given an oral dose of 250-280 mg ^{13}C-DHA, ^{13}C enrichment peaked at 2 h post-dose in plasma triglycerides when the tracer was given in the triglyceride form, but at 6 h post-dose when the tracer was esterified to phosphatidylcholine [69, 71]. Brossard *et al.* have reported a 1.4% apparent retro-conversion of ^{13}C-DHA to ^{13}C-docosapentaenoate (22:5 omega-3) and ^{13}C-EPA 3 d after giving the tracer [70]. These first results showed the feasibility of tracing DHA metabolism in humans. However, neither the impact of aging on ^{13}C-DHA metabolism nor its β-oxidation were investigated, although both may influence the somewhat higher blood levels of EPA and DHA commonly seen in healthy elderly [54, 65, 66, 68, 72]. Our group are pioneers in this field as we investigated the kinetics of ^{13}C-DHA in six young and six elderly participants [73]. We found that, in the elderly, ^{13}C-DHA was 4 times higher in plasma triglycerides and NEFA at 4 h post-dose, β-oxidation was 1.9 times higher, whereas apparent retro-conversion of ^{13}C-DHA to other ^{13}C-omega-3 fatty acids was 2.1 times higher 24 h and 7 d after tracer intake compared to the young adults [73]. Hence, because DHA seems to remain transiently for longer periods of time in the blood of the elderly compared to the young, it may thus indicate that efficiency to remove DHA from the blood is lower in the elderly than in the young, resulting in lower incorporation of DHA in the membrane of cells that serve to initiate signalization [65, 66, 68, 72]. This result is consistent with the transient slower metabolism of TG and LDL in older as compared to young adults and this was described in a previous section.

Our most recent work with tracers between old and young men was conducted with ^{13}C-EPA or arachidonic acid (^{13}C-ARA), two key fatty acids that are precursors of anti- and pro-inflammatory cytokines, respectively. Surprisingly, the kinetics of ^{13}C-EPA and ^{13}C-ARA was quite similar between young and old men despite a time x age interaction for ^{13}C-EPA kinetics where the postprandial shape

of the curve was steeper in old vs young men [74]. One intriguing result we obtained was that in old men, synthesis of DHA from EPA started 2 h after tracer intake whereas it was delayed to 1 d in young men. This result suggests that old adults might need more DHA than what was actually provided in their diet compared to the young men. However, newly synthesized DHA accumulated in the plasma of old men for 7 d and this might be because it remains for a longer period in the plasma as suggested by our previous study with ^{13}C-DHA. Therefore, there might be a defect in old adults to uptake DHA in the tissues. We also calculated that plasma half-life of ^{13}C-EPA was 2 d whereas that of ^{13}C-ARA was 4 d, similar to that of DHA. DHA and ARA are the two most concentrated long chain polyunsaturated fatty acids in brain membranes. With our β-oxidation measures using breath samples, we calculated ^{13}C-EPA whole-body half-life to be ~14 days in old men whereas in the younger group it was ~21 days [74]. This result indicates that older adults turn over EPA ~7 days faster than the younger adults. This is an intriguing result since epidemiological studies and results we obtained in previous studies [62, 65] support that old adults have twice as much plasma EPA, hence one would anticipate a lower whole-body turnover in old vs young adults. Therefore, it seems that there is somehow a disconnect between plasma levels of EPA and perhaps DHA and their kinetics, thus more studies are needed to understand the mechanism of these modifications and their possible consequences such as potential higher risk of cognitive decline.

3 Brain modifications during aging:

The brain is composed of 60% fat with one third of its content being ARA and DHA. The brain is hence the second most rich tissue in fat after adipose tissue. The brain fatty acids are however mostly PLs unlike the adipose tissue that is mainly composed of TGs. Because DHA is an important constituent of brain structure, there has been much interest in the association between the level of DHA in brain membranes, brain function and brain volume and losses during aging. Therefore, this section will summarize the evidence about morphological, functional, and content modifications of the brain during aging and whether dietary omega-3 intake can improve brain structure and function.

3.1. Morphological modifications of the brain during aging

There are a number of morphological modifications of the brain that occur during aging. Several studies have indicated that brain volume decreases over the course of the human lifespan. A review conducted by Hedman et al. [75] compiled the results of 56 longitudinal magnetic resonance imaging (MRI) studies on whole brain volumes in healthy individuals and concluded that the rate of total brain volume loss

is not constant throughout aging. For instance, the rate of brain volume loss after 35 years of age is approximately 0.2% per year. Between 35 and 60 years of age, the volume loss rate slowly increases to 0.5% followed by a steady volume loss of over 0.5% per year over 60 years of age [75]. Furthermore, other studies have indicated that volume loss in the whole brain is greater in males than in females [76, 77].

Several studies demonstrate a reduction of gray matter volume during aging [78-84]. More specifically, the volume of gray matter in the cortex and the cerebellum of older individuals is 18% and 13% smaller, respectively, than those of their younger counterparts [81]. There is also a significant loss of gray matter in the frontal, limbic, temporal, and parietal lobes but not in the occipital lobe [78, 83]. Similarly, studies have also indicated that there is a decrease of white matter volume in the brains of older individuals [81, 85-87]. According to Jäncke et al. [81], there is a decrease in white matter volume in the cortex and cerebellum of older individuals by 5% and ~9%, respectively, compared to younger adults. Moreover, one study indicated that the rate of decrease of white matter is not constant during aging [87]. Instead, white matter volume slowly increases before the age of 40, peaks at approximately 50 years of age, and then quickly decreases after the age of 60 [87]. As well, white matter hyperintensity lesions increase in size with age in the frontal, temporal, and parietal lobes but not in the occipital lobes [86].

In addition to age-related changes in the volume of the whole brain, gray matter, and white matter, there are also differences in the volume of specific brain structures. There seems to be a general decrease in the volume of the following brain structures in older individuals compared to younger individuals: cerebral hemisphere [76], frontal lobe [88, 89], parietal lobe [77, 88, 89], temporal lobe [88, 89], thalamus [81, 90], basal ganglia [89], and the cerebellum [89]. Notably, there is atrophy of the hippocampus during aging [77, 81, 91-93]. A meta-analysis by Fraser et al. [93] detailed hippocampal atrophy rates according to 28 studies. They determined that the overall rate of atrophy for the entire sample was 0.85% per year [93]. However, the rate of hippocampal atrophy reported in the studies differed based on mean age of the participants: rate of atrophy was 0.38% per year in studies with a mean age of 55, 0.98% per year for a mean age of 55 to 70 years, and 1.12% per year for a mean age of greater than 70 years. In contrast to the aforementioned structures, the ventricles of the brain increase in volume during aging [76, 91]. Altogether, there is generally good evidence supporting loss of matter in many brain structures, including loss in white and gray matter. These losses of brain matter can contribute to lower cognitive functions during aging.

3.2 Modification of brain functions during aging

In addition to the many structural changes that occur during aging, brain functions are also modified during this period. For instance, there is an age-related decrease in glucose metabolism in the whole brain and the frontal, parietal, and temporal lobes as well as in Broca's and Wernicke's areas [77]. It also seems that brain activation during the execution of motor functions is modified in older adults. For example, there is a decrease in blood-oxygen level dependent (BOLD) signals in multiple brain regions (sensorimotor cortex, cerebellum and thalamus) of older adults during mastication and an increase in BOLD signal in the prefrontal area [94]. Another study showed that classical motor coordination regions were activated during complex inter-limb coordination tasks, but that there was also increased activation of higher-level sensorimotor and frontal regions in older individuals [95]. Similarly, other studies have demonstrated that the performance of motor tasks result in increased activation of additional brain areas such as the basal ganglia, prefrontal cortex, precuneus, and the cerebellum [96-98] in older invividuals.

Moreover, cognitive functions are modified as a result of changes in the volume of various brain structures. For instance, a meta-analysis of 57 publications from the years 1984 to 1998 concluded that white matter hyperintensities are linked with poorer performance on cognitive tests for processing speed, immediate and delayed memory, executive functions, and global cognitive functioning [99]. Further, a decrease in the thalamus volume in older individuals is associated with attenuated performance on tests assessing cognitive speed [90]. An additional meta-analysis of 33 studies concluded that larger prefrontal cortex volume and thickness is correlated with better executive functioning [100]. In regard to hippocampus volume and memory, Van Petten [101] reported in a meta-analysis of 33 studies that the positive correlation between hippocampus size and episodic memory in older adults was weaker than expected. However, a more recent study demonstrated that smaller hippocampus size is significantly associated with lower performance in episodic memory, working memory, processing speed, and executive function tasks [102]. Similarly to motor function, it has been shown that older adults recruit additional brain regions during memory tasks [103-105].

Aging is also associated with changes in the activity of brain structures involved in sensation and perception. For instance, there are less areas activated in older versus younger adults in response to various odors [106]. A meta-analysis of 105 studies concluded that the activation of the fusiform gyrus, cerebellum, and hippocampus is elevated in elderly versus younger individuals during the processing of emotional faces [107]. Moreover, older individuals had greater activation of the prefrontal cortex during more difficult perceptual tasks compared to younger individuals [108]. The brains of older adults are also less responsive to blue light stimulation compared to younger adults [109].

More recent studies have shed light on the changes that occur in the functional neural networks of the brain. It seems that aging is associated with weaker connectivity in long-range connections and stronger connectivity of short-range connections [110, 111]. Elderly individuals also have less intra-network and greater inter-network connectivity [112, 113]. More specifically, older individuals have less connectivity within the default mode network (DMN) and somatomotor network [113], as well as greater connectivity between the salience network and the executive control network (ECN) and the DMN [112]. Moreover, age seems to shift dynamic functional connectivity from posterior to anterior regions, which is also reflected in the decreased activation of posterior regions during the decline of episodic memory in older individuals [114].

Overall, there are several morphological and functional modifications within the brain during aging and understanding how these modifications manifest could be helpful to limit the rate at which these declines occur.

3.3 Modifications of brain content during aging

The number of studies, particularly those that use neuroimaging techniques, that have evaluated the change in human brain content during aging is limited. Post-mortem examinations of the human brain have indicated that there is a change in protein and lipid content during aging. With regard to protein, one study indicated that there is a 5-15% decrease in total protein content of the brain between 30 and 90 years of age [115]. A decrease in protein content in the substantia nigra, hippocampus, caudate nucleus, and gray matter has also been reported [116, 117]. However, Söderberg et al. [116] found that protein content remained unchanged in the cerebellum, pons, and medulla oblongata of older individuals. Similarly, a number of post-mortem studies have demonstrated changes in the lipid content of older brains. For instance, Svenerholm et al. [118] reported that there is a linear decrease in cholesterol and phospholipids in the frontal and temporal cortices and a curvilinear decrease in cholesterol, PLs, cerebrosides, and sulfatides in frontal and temporal white matter between the ages of 20 and 100. In terms of PLs, Söderberg et al. [116] found that they were relatively unchanged during aging with only a 5-10% decrease in the oldest age group. A more recent study conducted by Hancock et al. [119] reported that PL content in the entorhinal cortex of older individuals is relatively stable during aging, but there is an increase in mitochondrial phosphatidylcholine (PC) and a decrease in mitochondrial phosphatidylethanolamine (PE). The same group reported that age is associated with an increase in mitochondrial PE containing DHA, but said the increase is not large enough to increase total DHA in the mitochondria. Norris et al. [120] examined phospholipid composition in the dorsolateral prefrontal cortex in individuals aged 20-100 years. They found that there is a general age-related increase in phospholipids containing DHA and decrease in PLs containing ARA and docosatetraenoic acid [120].

A recent study used positron emission tomography to assess the incorporation of DHA from plasma to the brain using carbon-11 ([1-C^{11}])-DHA in apolipoprotein E epsilon 4 allele (APOE4) carriers versus non-carriers [121]. APOE4 is the most important genetic risk of late-onset Alzheimer's disease [122]. Yassine et al. found that the mean global gray matter DHA incorporation coefficient was 16% higher in APOE4 carriers vs non-carriers [121]. A higher DHA incorporation coefficient was also observed in other regions including the entorhinal cortex [121]. However, the whole-brain DHA incorporation rate was not significantly different between APOE groups [121]. They also did not observe any age-related effects on DHA incorporation, but this may be due to the fact that only 4 of their 23 participants were over 50 years old [121]. The authors hypothesized that increased DHA incorporation in the brains of APOE4 carriers could be a compensatory mechanism to counteract brain DHA loss [121]. Our group also documented that the metabolism of DHA is imbalanced in APOE4 carriers [123-126] and that they are perhaps more vulnerable to DHA deficiency [127].

3.4 Does omega-3 fatty acid consumption improve brain structure and function?

There are a number of studies that have examined the relationship between omega-3 fatty acid consumption and brain structure and function. For instance, Gu et al. [128] evaluated the link between white matter integrity and dietary nutrient intake in 239 elderly participants. They assessed white matter integrity using fractional anisotropy measured by diffusion tensor imaging (DTI). They found that the nutrient pattern characterized by high consumption of omega-3 and omega-6 fatty acids and vitamin E was positively correlated with fractional anisotropy which corresponds to better white matter integrity [128]. Another group examined the relationship between dietary fish consumption and brain structural integrity in 260 cognitively normal adults aged 65 years or older [129]. Fish intake was measured using the National Cancer Institute Food Frequency Questionnaire and the gray matter volume of various brain regions was measured with MRI [129]. They found that eating baked or broiled fish weekly is positively associated with higher gray matter volume in several brain regions, including the hippocampus, posterior cingulate, precuneus, and the orbital frontal cortex [129]. Samieri and colleagues [130] evaluated the association between plasma EPA and DHA concentrations and gray matter atrophy in the medial temporal lobe in 281 individuals aged 65 years or older. The authors compared fatty acid plasma concentrations at baseline to the results of MRI examinations from baseline and four years after baseline [130]. They observed that greater levels of plasma EPA was associated with lower atrophy of the gray matter of the right amygdala and the hippocampal/parahippocampal region; this same association was not observed for plasma DHA levels [130], which is counterintuitive. Samieri et al. [130] also found that increased amygdala gray matter atrophy was linked with more depressive symptoms and poorer semantic memory performances compared to baseline. Lastly, Witte et al. [131] assessed the connection

between fish oil supplement consumption and brain structure and function in 65 participants aged 50 to 75 years. Participants consumed either fish oil, which contained 2.2 grams of omega-3 fatty acids, or a placebo daily for 26 weeks. Neuropsychological testing and MRI examinations were performed before and after the intervention period. The investigators found that after the 26-week intervention period, the fish oil group had better white matter structural integrity in selective white matter tracts in the frontal, temporal, parietal, and limbic areas [131]. They also observed that the fish oil group had significant increases in gray matter volume in the left hippocampus, precuneus, the superior temporal, inferior parietal and postcentral gyri, and in the right middle temporal gyrus [131]. In terms of performance on cognitive measures, they found that the fish oil group had an improvement of 26% on executive function scores compared to no improvement in the placebo group [131]. In addition, they found a positive correlation between verbal fluency scores and EPA percentage in red blood cell membranes in the fish oil group after intervention [131]. Although for many years it was thought that an intake of fish throughout life protects against cognitive decline, the recent evidence suggests that fish intake might not be required throughout life to improve brain structure and function. Hence, starting an EPA+DHA supplementation after 50 years old might benefit older individuals with respect to prevention of brain volume and function losses.

4 Are we ready for updated recommendations on dietary omega-3 fatty acids intake during aging?

In this review paper, we have outlined that there are many physiological modifications occurring during aging with respect to lipid metabolism and brain volume and function losses and that an omega-3 fatty acid intake might help to support the brain throughout aging. It is important to note that life expectancy is longer, which means that older adults may live longer with their chronic diseases. A major concern regarding old age is a decline in health, especially if this entails a loss of self-sufficiency and independence caused by a decline in cognition. A decline in working memory appears to be one of the major consequences of *normal* aging [132, 133]. As outlined in the previous sections, the brain undergoes physiological change during aging. While age is one risk of cognitive decline, this multifactorial disease is also increased by a complex interaction between both genetic and environmental risk factors [134-136].

We believe nutrition has a role to play in the prevention of cognitive decline but nutrition alone might not be as efficient as a multidomain intervention. Recent evidence from the FINGER trials [137] reported that combining physical exercise, personalized nutritional recommendations to avoid nutrient deficiencies, controlling cardiovascular risks and having cognitive stimulation prevented cognitive decline. However, they recently refocus their message by showing that dietary changes initiated early in the intervention was the most influential for global cognition improvement over two years of follow-up

[138]. Therefore, nutrition might have a key role to play in the prevention of cognitive decline. In the case of the FINGER study, dietary recommendations were not focussed on the consumption of fish oil but were either focused to alleviate nutritional deficiency including low blood levels of DHA. It also has to be emphasized that there is currently no drug to prevent, cure or delay the progression of dementia and that some pharmaceutical companies have shut down their research laboratories in this area. Therefore, prevention strategies are currently the most efficient means since once the disease process has started, there is no available drug for limiting its progression. However, there is one group in Canada working on a nutritional strategy, a ketogenic beverage. They reported that a ketogenic beverage increases brain energy metabolism in Alzheimer's patients [139, 140].

Returning to the question of if we are ready to change recommendations on omega-3 fatty acids, we think that we are not there yet. However, working on the biology of aging might greatly improve our understanding about what are «normal» modifications occurring during aging and what can become pathological. Seizing this opportunity, we might contribute to the prevention of cognitive decline in the future with nutrition playing a vital role in this process.

Acknowledgements

This work was supported by Canadian Institute of Health Research (CIHR, MOP 119454), Quebec Network of research in aging, Natural Science and Engineering Research Council of Canada, Research center on aging for a scholarship to LC, Faculté de Médecine et des sciences de la santé for a scholarship to HCM, and CIHR for a salary award to MP. MP also hold an institutional research chair on lipid metabolism during aging funded by the medical research center of Université de Sherbrooke.

Conflicts of Interest

MP received funding from Neptune Wellness Solutions but the funding did not funded studies cited in this review and did not funded any of the work of this review.

References

1. Barberger-Gateau P, Letenneur L, Deschamps V, Peres K, Dartigues JF, Renaud S. Fish, meat, and risk of dementia: cohort study. Bmj. 2002;325(7370):932-3.
2. Holub DJ, Holub BJ. Omega-3 fatty acids from fish oils and cardiovascular disease. Mol Cell Biochem. 2004;263(1-2):217-25.
3. Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS. Fish consumption and cognitive decline with age in a large community study. Arch Neurol. 2005;62(12):1849-53.
4. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr. 2006;83(6):S1467-76.
5. Vidgren HM, Agren JJ, Schwab U, Rissanen T, Hanninen O, Uusitupa MI. Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men. Lipids. 1997;32(7):697-705.
6. Calder PC. Polyunsaturated fatty acids and inflammation. Prostaglandins Leukot Essent Fatty Acids. 2006;75(3):197-202. Epub 2006/07/11.
7. Plourde M, Cunnane SC. Extremely limited synthesis of long chain polyunsaturates in adults: Implications for their dietary essentiality and use as suppements. Appl Physiol Nutr Metab. 2007;32(4):619-34.
8. Blasbalg TL, Hibbeln JR, Ramsden CE, Majchrzak SF, Rawlings RR. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. The American Journal of Clinical Nutrition. 2011;93(5):950-62.
9. Cunnane SC, Plourde M, Pifferi F, Begin M, Feart C, Barberger-Gateau P. Fish, docosahexaenoic acid and Alzheimer's disease. Prog Lipid Res. 2009;48(5):239-56. Epub 2009/04/14.
10. Salem N, Jr., Vandal M, Calon F. The benefit of docosahexaenoic acid for the adult brain in aging and dementia. Prostaglandins Leukot Essent Fatty Acids. 2015;92:15-22.
11. Barberger-Gateau P, Samieri C, Feart C, Plourde M. Dietary omega 3 polyunsaturated fatty acids and Alzheimer's disease: interaction with apolipoprotein E genotype. Curr Alzheimer Res. 2011;8(5):479-91.
12. Loef M, Walach H. The Omega-6/Omega-3 Ratio and Dementia or Cognitive Decline: A Systematic Review on Human Studies and Biological Evidence. Journal of Nutrition in Gerontology and Geriatrics. 2013;32(1):1-23.

13. van der Lee SJ, Teunissen CE, Pool R, Shipley MJ, Teumer A, Chouraki V, et al. Circulating metabolites and general cognitive ability and dementia: Evidence from 11 cohort studies. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2018;14(6):707-22. Epub 2018/01/10.
14. Mc Auley MT, Mooney KM. Computationally Modeling Lipid Metabolism and Aging: A Mini-review. Computational and structural biotechnology journal. 2015;13:38-46. Epub 2015/03/10.
15. Schwenk RW, Holloway GP, Luiken JJ, Bonen A, Glatz JF. Fatty acid transport across the cell membrane: regulation by fatty acid transporters. Prostaglandins, leukotrienes, and essential fatty acids. 2010;82(4-6):149-54. Epub 2010/03/09.
16. Mu H, Hoy CE. The digestion of dietary triacylglycerols. Prog Lipid Res. 2004;43(2):105-33.
17. Mattson FH, Volpenhein RA. The Digestion and Absorption of Triglycerides. J Biol Chem. 1964;239:2772-7.
18. Kunisaki S, C. M. Ultrasound growth patterns of fetal lung malformations: Implications on prenatal care and postnatal outcome. Prenat Diagn. 2015;35(24):89-90.
19. D'Aquila T, Hung YH, Carreiro A, Buhman KK. Recent discoveries on absorption of dietary fat: Presence, synthesis, and metabolism of cytoplasmic lipid droplets within enterocytes. Biochimica et biophysica acta. 2016;1861(8 Pt A):730-47. Epub 2016/04/25.
20. Bisgaier CL, Glickman RM. Intestinal synthesis, secretion, and transport of lipoproteins. Annu Rev Physiol. 1983;45:625-36.
21. Mansbach CM, 2nd, Nevin P. Intracellular movement of triacylglycerols in the intestine. J Lipid Res. 1998;39(5):963-8.
22. Cooper AD. Hepatic uptake of chylomicron remnants. J Lipid Res. 1997;38(11):2173-92.
23. Redgrave TG, Small DM. Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat. J Clin Invest. 1979;64(1):162-71.
24. Gruffat D, Durand D, Graulet B, Bauchart D. Regulation of VLDL synthesis and secretion in the liver. Reprod Nutr Dev. 1996;36(4):375-89.
25. Havel RJ. The formation of LDL: mechanisms and regulation. J Lipid Res. 1984;25(13):1570-6.
26. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science. 1986;232(4746):34-47.
27. Zhang Y, Ma KL, Ruan XZ, Liu BC. Dysregulation of the Low-Density Lipoprotein Receptor Pathway Is Involved in Lipid Disorder-Mediated Organ Injury. Int J Biol Sci. 2016;12(5):569-79.
28. B. B. Stress oxydant et pathologies cardiovasculaires. . Médecine Thérapeutique Cardiol. . 2006;2(1):43-52.
29. Fortier M, Tremblay-Mercier J, Plourde M, Chouinard-Watkins R, Vandal M, Pifferi F, et al. Higher plasma n-3 fatty acid status in the moderately healthy elderly in southern Quebec: higher fish intake or aging-related change in n-3 fatty acid metabolism? Prostaglandins, leukotrienes, and essential fatty acids. 2010;82(4-6):277-80. Epub 2010/03/09.
30. Einarsson K, Nilsell K, Leijd B, Angelin B. Influence of age on secretion of cholesterol and synthesis of bile acids by the liver. The New England journal of medicine. 1985;313(5):277-82. Epub 1985/08/01.
31. Verges B. Pathophysiology of diabetic dyslipidaemia: where are we? Diabetologia. 2015;58(5):886-99.
32. Austin MA, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. Am J Cardiol. 1998;81(4A):7B-12B.
33. Ericsson S, Berglund L, Frostegard J, Einarsson K, Angelin B. The influence of age on low density lipoprotein metabolism: effects of cholestyramine treatment in young and old healthy male subjects. J Intern Med. 1997;242(4):329-37.
34. Russell DW, Setchell KD. Bile acid biosynthesis. Biochemistry. 1992;31(20):4737-49.
35. Grundy SM, Cleeman JI, Rifkind BM, Kuller LH. Cholesterol lowering in the elderly population. Coordinating Committee of the National Cholesterol Education Program. Arch Intern Med. 1999;159(15):1670-8.
36. Fernandez ML, West KL. Mechanisms by which dietary fatty acids modulate plasma lipids. J Nutr. 2005;135(9):2075-8.
37. Block RC, Harris WS, Pottala JV. Determinants of Blood Cell Omega-3 Fatty Acid Content. The open biomarkers journal. 2008;1:1-6.

38. Gellert S, Schuchardt JP, Hahn A. Low long chain omega-3 fatty acid status in middle-aged women. Prostaglandins Leukot Essent Fatty Acids. 2017;117:54-9.
39. Harris WS, Luo J, Pottala JV, Espeland MA, Margolis KL, Manson JE, et al. Red blood cell polyunsaturated fatty acids and mortality in the Women's Health Initiative Memory Study. J Clin Lipidol. 2017;11(1):250-9 e5.
40. Harris WS, Pottala JV, Lacey SM, Vasan RS, Larson MG, Robins SJ. Clinical correlates and heritability of erythrocyte eicosapentaenoic and docosahexaenoic acid content in the Framingham Heart Study. Atherosclerosis. 2012;225(2):425-31.
41. Harris WS, Pottala JV, Varvel SA, Borowski JJ, Ward JN, McConnell JP. Erythrocyte omega-3 fatty acids increase and linoleic acid decreases with age: observations from 160,000 patients. Prostaglandins Leukot Essent Fatty Acids. 2013;88(4):257-63.
42. Sands SA, Reid KJ, Windsor SL, Harris WS. The impact of age, body mass index, and fish intake on the EPA and DHA content of human erythrocytes. Lipids. 2005;40(4):343-7.
43. Aarsetoey H, Ponitz V, Grundt H, Staines H, Harris WS, Nilsen DW. (n-3) Fatty acid content of red blood cells does not predict risk of future cardiovascular events following an acute coronary syndrome. J Nutr. 2009;139(3):507-13.
44. Block RC, Harris WS, Reid KJ, Sands SA, Spertus JA. EPA and DHA in blood cell membranes from acute coronary syndrome patients and controls. Atherosclerosis. 2008;197(2):821-8.
45. Caprari P, Scuteri A, Salvati AM, Bauco C, Cantafora A, Masella R, et al. Aging and red blood cell membrane: a study of centenarians. Exp Gerontol. 1999;34(1):47-57.
46. Carver JD, Benford VJ, Han B, Cantor AB. The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects. Brain Res Bull. 2001;56(2):79-85.
47. Farzaneh-Far R, Harris WS, Garg S, Na B, Whooley MA. Inverse association of erythrocyte n-3 fatty acid levels with inflammatory biomarkers in patients with stable coronary artery disease: The Heart and Soul Study. Atherosclerosis. 2009;205(2):538-43.
48. Itomura M, Fujioka S, Hamazaki K, Kobayashi K, Nagasawa T, Sawazaki S, et al. Factors influencing EPA+DHA levels in red blood cells in Japan. In vivo. 2008;22(1):131-5.
49. Thorlaksdottir AY, Skuladottir GV, Petursdottir AL, Tryggvadottir L, Ogmundsdottir HM, Eyfjord JE, et al. Positive association between plasma antioxidant capacity and n-3 PUFA in red blood cells from women. Lipids. 2006;41(2):119-25.
50. Yanagisawa N, Shimada K, Miyazaki T, Kume A, Kitamura Y, Ichikawa R, et al. Polyunsaturated fatty acid levels of serum and red blood cells in apparently healthy Japanese subjects living in an urban area. Journal of atherosclerosis and thrombosis. 2010;17(3):285-94.
51. Brenna JT, Plourde M, Stark KD, Jones PJ, Lin YH. Best practices for the design, laboratory analysis, and reporting of trials involving fatty acids. Am J Clin Nutr. 2018;108(2):211-27.
52. Asciutti-Moura LS, Guilland JC, Fuchs F, Richard D, Klepping J. Fatty acid composition of serum lipids and its relation to diet in an elderly institutionalized population. Am J Clin Nutr. 1988;48(4):980-7.
53. Crowe FL, Skeaff CM, Green TJ, Gray AR. Serum n-3 long-chain PUFA differ by sex and age in a population-based survey of New Zealand adolescents and adults. Br J Nutr. 2008;99(1):168-74.
54. de Groot RH, van Boxtel MP, Schiepers OJ, Hornstra G, Jolles J. Age dependence of plasma phospholipid fatty acid levels: potential role of linoleic acid in the age-associated increase in docosahexaenoic acid and eicosapentaenoic acid concentrations. Br J Nutr. 2009;102(7):1058-64.
55. Dewailly E, Blanchet C, Gingras S, Lemieux S, Holub BJ. Cardiovascular disease risk factors and n-3 fatty acid status in the adult population of James Bay Cree. Am J Clin Nutr. 2002;76(1):85-92.
56. Dewailly E, Blanchet C, Lemieux S, Sauve L, Gingras S, Ayotte P, et al. n-3 Fatty acids and cardiovascular disease risk factors among the Inuit of Nunavik. Am J Clin Nutr. 2001;74(4):464-73.
57. Dewailly EE, Blanchet C, Gingras S, Lemieux S, Sauve L, Bergeron J, et al. Relations between n-3 fatty acid status and cardiovascular disease risk factors among Quebecers. Am J Clin Nutr. 2001;74(5):603-11.
58. Ogura T, Takada H, Okuno M, Kitade H, Matsuura T, Kwon M, et al. Fatty acid composition of plasma, erythrocytes and adipose: their correlations and effects of age and sex. Lipids. 2010;45(2):137-44.

578 59. Flock MR, Skulas-Ray AC, Harris WS, Etherton TD, Fleming JA, Kris-Etherton PM. Determinants of
579 erythrocyte omega-3 fatty acid content in response to fish oil supplementation: a dose-response randomized
580 controlled trial. Journal of the American Heart Association. 2013;2(6):e000513.
581 60. Witt PM, Christensen JH, Ewertz M, Aardestrup IV, Schmidt EB. The incorporation of marine n-3 PUFA into
582 platelets and adipose tissue in pre- and postmenopausal women: a randomised, double-blind, placebo-controlled
583 trial. Br J Nutr. 2010;104(3):318-25.
584 61. Vandal M, Freemantle E, Tremblay-Mercier J, Plourde M, Fortier M, Bruneau J, et al. Plasma omega-3 fatty
585 acid response to a fish oil supplement in the healthy elderly. Lipids. 2008;43(11):1085-9.
586 62. Fortier M, Tremblay-Mercier J, Plourde M, Chouinard-Watkins R, Vandal M, Pifferi F, et al. Higher plasma
587 n-3 fatty acid status in the moderately healthy elderly in southern Quebec: higher fish intake or aging-related
588 change in n-3 fatty acid metabolism? Prostaglandins Leukot Essent Fatty Acids. 2010;82(4-6):277-80.
589 63. Meydani M, Natiello F, Goldin B, Free N, Woods M, Schaefer E, et al. Effect of long-term fish oil
590 supplementation on vitamin E status and lipid peroxidation in women. J Nutr. 1991;121(4):484-91.
591 64. Meydani SN, Endres S, Woods MM, Goldin BR, Soo C, Morrill-Labrode A, et al. Oral (n-3) fatty acid
592 supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and
593 older women. J Nutr. 1991;121(4):547-55.
594 65. Plourde M, Tremblay-Mercier J, Fortier M, Pifferi F, Cunnane SC. Eicosapentaenoic acid decreases
595 postprandial beta-hydroxybutyrate and free fatty acid responses in healthy young and elderly. Nutrition.
596 2009;25(3):289-94.
597 66. Rees D, Miles EA, Banerjee T, Wells SJ, Roynette CE, Wahle KW, et al. Dose-related effects of
598 eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. Am J
599 Clin Nutr. 2006;83(2):331-42.
600 67. Walker CG, Browning LM, Mander AP, Madden J, West AL, Calder PC, et al. Age and sex differences in the
601 incorporation of EPA and DHA into plasma fractions, cells and adipose tissue in humans. Br J Nutr.
602 2014;111(4):679-89.
603 68. Vandal M, Freemantle E, Tremblay-Mercier J, Plourde M, Fortier M, Bruneau J, et al. Plasma omega-3 fatty
604 acid response to a fish oil supplement in the healthy elderly. Lipids. 2008;43(11):1085-9.
605 69. Brossard N, Croset M, Normand S, Pousin J, Lecerf J, Laville M, et al. Human plasma albumin transports
606 [13C]docosahexaenoic acid in two lipid forms to blood cells. J Lipid Res. 1997;38(8):1571-82.
607 70. Brossard N, Croset M, Pachiaudi C, Riou JP, Tayot JL, Lagarde M. Retroconversion and metabolism of
608 [13C]22:6n-3 in humans and rats after intake of a single dose of [13C]22:6n-3-triacylglycerols. Am J Clin Nutr.
609 1996;64(4):577-86.
610 71. Lemaitre-Delaunay D, Pachiaudi C, Laville M, Pousin J, Armstrong M, Lagarde M. Blood compartmental
611 metabolism of docosahexaenoic acid (DHA) in humans after ingestion of a single dose of [(^{13})C]DHA in
612 phosphatidylcholine. J Lipid Res. 1999;40(10):1867-74.
613 72. Fortier M, Tremblay-Mercier J, Plourde M, Chouinard-Watkins R, Vandal M, Pifferi F, et al. Higher plasma
614 n-3 fatty acid status in the moderately healthy elderly in southern Quebec: higher fish intake or aging-related
615 change in n-3 fatty acid metabolism? Prostaglandins Leukot Essent Fatty Acids. ;82(4-6):277-80. Epub 2010/03/09.
616 73. Plourde M, Chouinard-Watkins R, Vandal M, Zhang Y, Lawrence P, Brenna JT, et al. Plasma incorporation,
617 apparent retroconversion and beta-oxidation of 13C-docosahexaenoic acid in the elderly. Nutrition & metabolism.
618 2011;8:5. Epub 2011/01/29.
619 74. Leveille P, Chouinard-Watkins R, Windust A, Lawrence P, Cunnane SC, Brenna JT, et al. Metabolism of
620 uniformly labeled 13C-eicosapentaenoic acid and 13C-arachidonic acid in young and old men. Am J Clin Nutr.
621 2017.
622 75. Hedman AM, van Haren NE, Schnack HG, Kahn RS, Hulshoff Pol HE. Human brain changes across the life
623 span: a review of 56 longitudinal magnetic resonance imaging studies. Hum Brain Mapp. 2012;33(8):1987-2002.
624 76. Coffey CE, Lucke JF, Saxton JA, Ratcliff G, Unitas LJ, Billig B, et al. Sex differences in brain aging: a
625 quantitative magnetic resonance imaging study. Arch Neurol. 1998;55(2):169-79.

626 77. Murphy DG, DeCarli C, McIntosh AR, Daly E, Mentis MJ, Pietrini P, et al. Sex differences in human brain
627 morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission
628 tomography study on the effect of aging. Arch Gen Psychiatry. 1996;53(7):585-94.
629 78. Bourisly AK, El-Beltagi A, Cherian J, Gejo G, Al-Jazzaf A, Ismail M. A voxel-based morphometric magnetic
630 resonance imaging study of the brain detects age-related gray matter volume changes in healthy subjects of 21-45
631 years old. The neuroradiology journal. 2015;28(5):450-9.
632 79. Draganski B, Ashburner J, Hutton C, Kherif F, Frackowiak RS, Helms G, et al. Regional specificity of MRI
633 contrast parameter changes in normal ageing revealed by voxel-based quantification (VBQ). Neuroimage.
634 2011;55(4):1423-34.
635 80. Hafkemeijer A, Altmann-Schneider I, de Craen AJ, Slagboom PE, van der Grond J, Rombouts SA.
636 Associations between age and gray matter volume in anatomical brain networks in middle-aged to older adults.
637 Aging Cell. 2014;13(6):1068-74.
638 81. Jancke L, Merillat S, Liem F, Hanggi J. Brain size, sex, and the aging brain. Hum Brain Mapp.
639 2015;36(1):150-69.
640 82. Minkova L, Habich A, Peter J, Kaller CP, Eickhoff SB, Kloppel S. Gray matter asymmetries in aging and
641 neurodegeneration: A review and meta-analysis. Hum Brain Mapp. 2017;38(12):5890-904.
642 83. Peng F, Wang L, Geng Z, Zhu Q, Song Z. A Cross-Sectional Voxel-Based Morphometric Study of Age- and
643 Sex-Related Changes in Gray Matter Volume in the Normal Aging Brain. Journal of computer assisted tomography.
644 2016;40(2):307-15.
645 84. Tremblay P, Dick AS, Small SL. Functional and structural aging of the speech sensorimotor neural system:
646 functional magnetic resonance imaging evidence. Neurobiol Aging. 2013;34(8):1935-51.
647 85. Callaghan MF, Freund P, Draganski B, Anderson E, Cappelletti M, Chowdhury R, et al. Widespread age-
648 related differences in the human brain microstructure revealed by quantitative magnetic resonance imaging.
649 Neurobiol Aging. 2014;35(8):1862-72.
650 86. Honda Y, Noguchi A, Maruyama K, Tamura A, Saito I, Sei K, et al. Volumetric analyses of cerebral white
651 matter hyperintensity lesions on magnetic resonance imaging in a Japanese population undergoing medical check-
652 up. Geriatrics & gerontology international. 2015;15 Suppl 1:43-7.
653 87. Liu H, Wang L, Geng Z, Zhu Q, Song Z, Chang R, et al. A voxel-based morphometric study of age- and sex-
654 related changes in white matter volume in the normal aging brain. Neuropsychiatric disease and treatment.
655 2016;12:453-65.
656 88. Coffler MS, Patel K, Dahan MH, Yoo RY, Malcom PJ, Chang RJ. Enhanced granulosa cell responsiveness to
657 follicle-stimulating hormone during insulin infusion in women with polycystic ovary syndrome treated with
658 pioglitazone. J Clin Endocrinol Metab. 2003;88(12):5624-31. Epub 2003/12/13.
659 89. Xu J, Kobayashi S, Yamaguchi S, Iijima K, Okada K, Yamashita K. Gender effects on age-related changes in
660 brain structure. AJNR Am J Neuroradiol. 2000;21(1):112-8.
661 90. Van Der Werf YD, Tisserand DJ, Visser PJ, Hofman PA, Vuurman E, Uylings HB, et al. Thalamic volume
662 predicts performance on tests of cognitive speed and decreases in healthy aging. A magnetic resonance imaging-
663 based volumetric analysis. Brain research. Cognitive brain research. 2001;11(3):377-85.
664 91. Erten-Lyons D, Dodge HH, Woltjer R, Silbert LC, Howieson DB, Kramer P, et al. Neuropathologic basis of
665 age-associated brain atrophy. JAMA Neurol. 2013;70(5):616-22.
666 92. Fjell AM, Westlye LT, Grydeland H, Amlien I, Espeseth T, Reinvang I, et al. Critical ages in the life course of
667 the adult brain: nonlinear subcortical aging. Neurobiol Aging. 2013;34(10):2239-47.
668 93. Fraser MA, Shaw ME, Cherbuin N. A systematic review and meta-analysis of longitudinal hippocampal
669 atrophy in healthy human ageing. Neuroimage. 2015;112:364-74.
670 94. Onozuka M, Fujita M, Watanabe K, Hirano Y, Niwa M, Nishiyama K, et al. Age-related changes in brain
671 regional activity during chewing: a functional magnetic resonance imaging study. Journal of dental research.
672 2003;82(8):657-60.
673 95. Heuninckx S, Wenderoth N, Swinnen SP. Systems neuroplasticity in the aging brain: recruiting additional
674 neural resources for successful motor performance in elderly persons. J Neurosci. 2008;28(1):91-9.

96. Holtzer R, Epstein N, Mahoney JR, Izzetoglu M, Blumen HM. Neuroimaging of mobility in aging: a targeted review. The journals of gerontology. Series A, Biological sciences and medical sciences. 2014;69(11):1375-88.
97. Kim JH, Lee YS, Lee JJ, Song HJ, Yoo DS, Lee HJ, et al. Functional magnetic resonance imaging reveals age-related alterations to motor networks in weighted elbow flexion-extension movement. Neurological research. 2010;32(9):995-1001.
98. Linortner P, Jehna M, Johansen-Berg H, Matthews P, Schmidt R, Fazekas F, et al. Aging associated changes in the motor control of ankle movements in the brain. Neurobiol Aging. 2014;35(10):2222-9.
99. Gunning-Dixon FM, Raz N. The cognitive correlates of white matter abnormalities in normal aging: a quantitative review. Neuropsychology. 2000;14(2):224-32.
100. Yuan P, Raz N. Prefrontal cortex and executive functions in healthy adults: a meta-analysis of structural neuroimaging studies. Neurosci Biobehav Rev. 2014;42:180-92.
101. Van Petten C. Relationship between hippocampal volume and memory ability in healthy individuals across the lifespan: review and meta-analysis. Neuropsychologia. 2004;42(10):1394-413.
102. O'Shea A, Cohen RA, Porges EC, Nissim NR, Woods AJ. Cognitive Aging and the Hippocampus in Older Adults. Front Aging Neurosci. 2016;8:298.
103. Gonneaud J, Lecouvey G, Groussard M, Gaubert M, Landeau B, Mezenge F, et al. Functional dedifferentiation and reduced task-related deactivations underlie the age-related decline of prospective memory. Brain imaging and behavior. 2017;11(6):1873-84.
104. Meusel LA, Grady CL, Ebert PE, Anderson ND. Brain-behavior relationships in source memory: Effects of age and memory ability. Cortex. 2017;91:221-33.
105. Rieckmann A, Pudas S, Nyberg L. Longitudinal Changes in Component Processes of Working Memory. eNeuro. 2017;4(2).
106. Suzuki Y, Critchley HD, Suckling J, Fukuda R, Williams SC, Andrew C, et al. Functional magnetic resonance imaging of odor identification: the effect of aging. The journals of gerontology. Series A, Biological sciences and medical sciences. 2001;56(12):M756-60.
107. Fusar-Poli P, Placentino A, Carletti F, Landi P, Allen P, Surguladze S, et al. Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. Journal of psychiatry & neuroscience : JPN. 2009;34(6):418-32.
108. Grady CL. Age-related differences in face processing: a meta-analysis of three functional neuroimaging experiments. Canadian journal of experimental psychology = Revue canadienne de psychologie experimentale. 2002;56(3):208-20.
109. Daneault V, Hebert M, Albouy G, Doyon J, Dumont M, Carrier J, et al. Aging reduces the stimulating effect of blue light on cognitive brain functions. Sleep. 2014;37(1):85-96.
110. Sala-Llonch R, Junque C, Arenaza-Urquijo EM, Vidal-Pineiro D, Valls-Pedret C, Palacios EM, et al. Changes in whole-brain functional networks and memory performance in aging. Neurobiol Aging. 2014;35(10):2193-202.
111. Tomasi D, Volkow ND. Aging and functional brain networks. Molecular psychiatry. 2012;17(5):471, 549-58.
112. Archer JA, Lee A, Qiu A, Chen SH. A Comprehensive Analysis of Connectivity and Aging Over the Adult Life Span. Brain connectivity. 2016;6(2):169-85.
113. Geerligs L, Maurits NM, Renken RJ, Lorist MM. Reduced specificity of functional connectivity in the aging brain during task performance. Hum Brain Mapp. 2014;35(1):319-30.
114. Zhang H, Lee A, Qiu A. A posterior-to-anterior shift of brain functional dynamics in aging. Brain structure & function. 2017;222(8):3665-76.
115. Naber D, Dahnke HG. Protein and nucleic acid content in the aging human brain. Neuropathology and applied neurobiology. 1979;5(1):17-24.
116. Soderberg M, Edlund C, Kristensson K, Dallner G. Lipid compositions of different regions of the human brain during aging. J Neurochem. 1990;54(2):415-23. Epub 1990/02/01.
117. Surowka AD, Adamek D, Radwanska E, Szczerbowska-Boruchowska M. Variability of protein and lipid composition of human subtantia nigra in aging: Fourier transform infrared microspectroscopy study. Neurochem Int. 2014;76:12-22.

724 118. Svennerholm L, Bostrom K, Jungbjer B, Olsson L. Membrane lipids of adult human brain: lipid composition
725 of frontal and temporal lobe in subjects of age 20 to 100 years. J Neurochem. 1994;63(5):1802-11. Epub
726 1994/11/01.
727 119. Hancock SE, Friedrich MG, Mitchell TW, Truscott RJ, Else PL. The phospholipid composition of the human
728 entorhinal cortex remains relatively stable over 80 years of adult aging. GeroScience. 2017;39(1):73-82.
729 120. Norris SE, Friedrich MG, Mitchell TW, Truscott RJW, Else PL. Human prefrontal cortex phospholipids
730 containing docosahexaenoic acid increase during normal adult aging, whereas those containing arachidonic acid
731 decrease. Neurobiol Aging. 2015;36(4):1659-69.
732 121. Yassine HN, Croteau E, Rawat V, Hibbeln JR, Rapoport SI, Cunnane SC, et al. DHA brain uptake and APOE4
733 status: a PET study with [1-(11)C]-DHA. Alzheimers Res Ther. 2017;9(1):23.
734 122. Coon KD, Myers AJ, Craig DW, Webster JA, Pearson JV, Lince DH, et al. A high-density whole-genome
735 association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease. J
736 Clin Psychiatry. 2007;68(4):613-8.
737 123. Chouinard-Watkins R, Conway V, Minihane AM, Jackson KG, Lovegrove JA, Plourde M. Interactive impact
738 of BMI and APOE genotype on the plasma long chain polyunsaturated fatty acid response to a fish oil supplement
739 in healthy participants Am J Clin Nutr. 2015;102:505-13.
740 124. Chouinard-Watkins R, Rioux-Perreault C, Fortier M, Tremblay-Mercier J, Zhang Y, Lawrence P, et al.
741 Disturbance in uniformly 13C-labelled DHA metabolism in elderly human subjects carrying the apoE epsilon4
742 allele. Br J Nutr. 2013;110(10):1751-9. Epub 2013/05/02.
743 125. Conway V, Allard MJ, Minihane AM, Jackson KG, Lovegrove JA, Plourde M. Postprandial enrichment of
744 triacylglycerol-rich lipoproteins with omega-3 fatty acids: lack of an interaction with apolipoprotein E genotype?
745 Lipids Health Dis. 2014;13(1):148. Epub 2014/09/18.
746 126. Plourde M, Vohl MC, Vandal M, Couture P, Lemieux S, Cunnane SC. Plasma n-3 fatty acid response to an n-
747 3 fatty acid supplement is modulated by apoE epsilon4 but not by the common PPAR-alpha L162V polymorphism
748 in men. Br J Nutr. 2009;102(8):1121-4. Epub 2009/10/16.
749 127. Nock TG, Chouinard-Watkins R, Plourde M. Carriers of an apolipoprotein E epsilon 4 allele are more
750 vulnerable to a dietary deficiency in omega-3 fatty acids and cognitive decline. Biochim Biophys Acta.
751 2017;1862(10 Pt A):1068-78.
752 128. Gu Y, Vorburger RS, Gazes Y, Habeck CG, Stern Y, Luchsinger JA, et al. White matter integrity as a mediator
753 in the relationship between dietary nutrients and cognition in the elderly. Ann Neurol. 2016;79(6):1014-25.
754 129. Raji CA, Erickson KI, Lopez OL, Kuller LH, Gach HM, Thompson PM, et al. Regular fish consumption and age-
755 related brain gray matter loss. American journal of preventive medicine. 2014;47(4):444-51.
756 130. Samieri C, Maillard P, Crivello F, Proust-Lima C, Peuchant E, Helmer C, et al. Plasma long-chain omega-3
757 fatty acids and atrophy of the medial temporal lobe. Neurology. 2012;79(7):642-50.
758 131. Witte AV, Kerti L, Hermannstadter HM, Fiebach JB, Schreiber SJ, Schuchardt JP, et al. Long-chain omega-3
759 fatty acids improve brain function and structure in older adults. Cereb Cortex. 2014;24(11):3059-68.
760 132. Salthouse TA, Mitchell DR, Palmon R. Memory and age differences in spatial manipulation ability. Psychol
761 Aging. 1989;4(4):480-6. Epub 1989/12/01.
762 133. Salthouse TA, Mitchell DR, Skovronek E, Babcock RL. Effects of adult age and working memory on
763 reasoning and spatial abilities. J Exp Psychol Learn Mem Cogn. 1989;15(3):507-16. Epub 1989/05/01.
764 134. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. Lancet. 2006;368(9533):387-403.
765 135. Deary IJ, Whiteman MC, Pattie A, Starr JM, Hayward C, Wright AF, et al. Cognitive change and the APOE
766 epsilon 4 allele. Nature. 2002;418(6901):932.
767 136. Hoyer S. The aging brain. Changes in the neuronal insulin/insulin receptor signal transduction cascade
768 trigger late-onset sporadic Alzheimer disease (SAD). A mini-review. J Neural Transm. 2002;109(7-8):991-1002.
769 137. Rosenberg A, Ngandu T, Rusanen M, Antikainen R, Backman L, Havulinna S, et al. Multidomain lifestyle
770 intervention benefits a large elderly population at risk for cognitive decline and dementia regardless of baseline
771 characteristics: The FINGER trial. Alzheimers Dement. 2018;14(3):263-70.

772 138. Lehtisalo J, Levalahti E, Lindstrom J, Hanninen T, Paajanen T, Peltonen M, et al. Dietary changes and
773 cognition over 2 years within a multidomain intervention trial-The Finnish Geriatric Intervention Study to Prevent
774 Cognitive Impairment and Disability (FINGER). Alzheimers Dement. 2018.
775 139. Croteau E, Castellano CA, Fortier M, Bocti C, Fulop T, Paquet N, et al. A cross-sectional comparison of brain
776 glucose and ketone metabolism in cognitively healthy older adults, mild cognitive impairment and early
777 Alzheimer's disease. Exp Gerontol. 2018;107:18-26.
778 140. Croteau E, Castellano CA, Richard MA, Fortier M, Nugent S, Lepage M, et al. Ketogenic Medium Chain
779 Triglycerides Increase Brain Energy Metabolism in Alzheimer's Disease. J Alzheimers Dis. 2018;64(2):551-61.
780 141. Nogi A, Yang J, Li L, Yamasaki M, Watanabe M, Hashimoto M, et al. Plasma n-3 polyunsaturated fatty acid
781 and cardiovascular disease risk factors in Japanese, Korean and Mongolian workers. Journal of occupational
782 health. 2007;49(3):205-16.
783 142. Skuladottir GV, Gudmundsdottir S, Olafsson GB, Sigurdsson SB, Sigfusson N, Axelsson J. Plasma fatty acids
784 and lipids in two separate, but genetically comparable, Icelandic populations. Lipids. 1995;30(7):649-55.
785 143. Stark KD, Park EJ, Holub BJ. Fatty acid composition of serum phospholipid of premenopausal women and
786 postmenopausal women receiving and not receiving hormone replacement therapy. Menopause. 2003;10(5):448-
787 55.
788 144. Stark KD, Beblo S, Murthy M, Whitty JE, Buda-Abela M, Janisse J, et al. Alcohol consumption in pregnant,
789 black women is associated with decreased plasma and erythrocyte docosahexaenoic acid. Alcoholism, clinical and
790 experimental research. 2005;29(1):130-40.
791 145. Yesilyurt B, Whittingstall K, Ugurbil K, Logothetis NK, Uludag K. Relationship of the BOLD signal with VEP
792 for ultrashort duration visual stimuli (0.1 to 5 ms) in humans. J Cereb Blood Flow Metab. 2010;30(2):449-58. Epub
793 2009/10/22.

794

795 Table 1: Cross-sectional modulation by age of blood fatty acid

				Age-increasing effects at baseline in blood pool		
Reference	***n*, sex and age**	**Blood pool**	**Age-increasing effects**	**Omega-3 index**	**EPA**	**DHA**
[43]	n=460, 299 men and 161 women, 29-97 y (~72 y)	RBC	9.8 y older in Higher Omega-3 Index Quartile compared to Lower Quartile	Higher Omega-3 Index quartile were 9.8 y older vs lower quartile		
[52]	53 institutionalized elderly subjects (24 men and 29 women), ≥ 60 y (~ 79 y); 24 young healthy adults, 20 – 42 y (~29 y)	Plasma NEFA, TG, CE and PL	In plasma PL: EPA higher in elderly; DHA and DPA: appear lower in the elderly but non-significantly different		PL: 2.1 fold higher	
[44]	768 acute coronary syndrome patients and 768 matched controls (66 % male, ~61 y)	RBC membranes	Positive relation between age and EPA and DHA levels: 8 years older in those with higher EPA + DHA levels vs those with lower group		Higher RBC EPA + DHA group: 8 y older compared to Lower group	Higher RBC EPA + DHA group: 8 y older compared to Lower group
[37]	704 outpatients (67% male), ~62 y	RBC	RBC Omega-3 Index increases with age	5.3% increase by 10 years increase		
[45]	15 centenarians (12 females and 3 males),~ 103 y (101–107 y), living in a family unit, self-sufficient and without major illnesses and 13 normal subjects (6 males and 7 females), ~65 y (6.0 – 69 y)	RBC-PL	Increased DHA in RBC-PC and in RBC-PE, and increased DPA in RBC-PS and RBC-PE;			PC: 2.2 fold higher PE: 1.6 fold higher
[53]	2793 New Zealanders ≥15 y (men and women)	Serum PL, CE and TG	Serum PL: EPA and DHA increase with age in both sexes while DPA increases with age only in women aged between of 20 and 73 y		PL: in both sexes, increased by 0.3 mol% between 20 and 73 y	PL: in both sexes, increased by 0.3 mol% between 20 and 73 y

[54]	234 men and women (Dutch: low fish consumption), 36 to 88 years (~60 y)	Plasma PL	Significant positive relationship between age and plasma PL concentrations of DHA and EPA.		PL: ~1.5 fold increased between 36 to 88 y	PL: ~1.3 fold increased between 36 to 88 y
[56]	426 Inuits, 18 to 74 years: 179 men (~38.7 y) and 247 women (~37.8 y), n=254 in 18-39 y and n=172 in ≥40 y	Plasma PL	Concentrations of EPA, DHA and EPA + DHA increased significantly with age		2.4 fold higher in ≥40 y group compared to 18-39 y group	1.4 fold higher in ≥40 y group compared to 18-39 y group
[57]	1460 subjects, 18–74 years: 722 men (~40.6 y) and 738 women (~39.6 y), n=784 in 18-34 y, n=432 in 35-49 y and n=244 in 50-74 y	Plasma PL	Older persons had higher EPA, DHA, EPA+DHA, EPA: AA and n-3: n-6 ratio in older vs younger individuals		1.1 fold higher in 50-74 y compared to 18-34 y	1.2 fold higher in 50-74 y compared to 18-34 y
[55]	917 subjects, 18-74 y: 422 men (~36.0 y) and 495 women (~35.6 y), n=536 in 18-34 y, n=220 in 35-49 y and n=161 in 50-74 y	Plasma PL	EPA: AA, n-3: n-6 FA, and concentrations of EPA, DHA, and EPA+DHA did not vary according to sex, but there was a significant increase in the concentrations with age		2.5 fold higher in 50-74 y compared to 18-34 y	1.7 fold higher in 50-74 y compared to 18-34 y
[47]	992 participants (mainly men: >80%), age: early 50s to late 70s	RBC membranes	Lower levels EPA + DHA were significantly associated with younger age			
[38]	446 women, ~48,5 y (40–60 y)	RBC membrane	In women aged ≥50 years, EPA and DPA levels and omega-3 index were significantly higher compared to women under the age of 50 years.	4% higher in ≥50 y compared to <50 y	13% higher in ≥50 y compared to <50 y	
[40]	n= 3196, 55 % women, ~66 y (40-74 y)	RBC	RBC Omega-3 Index increases with age	5% increase every decade		

[41]	159 771 patients (48% males, 52% females) being evaluated by their physicians for CVD risk	RBC	Increases in EPA and DHA each decade. After age 70, significant decrease in EPA while DHA remain high	7% increase by decade until 70 y, stable thereafter	13% increase by decade up to 70 y, then 9% decrease by decade	6% increase by decade until 70 y, stable thereafter
[39]	6501 women aged 65–80, ~15 years follow-up	RBC	RBC Omega-3 Index increases with age:	Higher Omega-3 index quartile: 0.6 y older compared to lower quartile		
[48]	n=456, 320 men and 136 women, 18 to 70 y (~42.5 y)	RBC-PL	EPA+DHA: ~1.4 fold increase in both gender between 18-20 vs 60+ years			
[141]	411 Japaneses (194 men and 217 women), 418 Koreans (240 men and 178 women) and 252 Mongolians (100 men and 152 women) aged 30-60 y	Plasma	EPA and DHA increase with age in Japanese and Koreans.		Japaneses: 1.4 fold increase Koreans: 1.9 fold increase	Japaneses: 1.2 fold increase Koreans: 1.3 fold increase Mongolians: 1.1 fold decrease
[58]	75 adults admitted for elective surgery: 48 men (~58 y: 27-81 y) and 27 women (~58 y: 33-74 y)	Plasma PL, RBC-PL and AT	Positive correlation between EPA+DHA and age, in plasma and RBC-PL but not in AT			
[42]	163 adults, 74 men and 89 women, 20 to 80 years	RBC	Omega-3 Index increased each decade but decreased by 0.3 units with each 3-unit increase in BMI	0.5 unit increase by 10 years of age		
[142]	119 subjects for each population, Icelandic (59 males and 60 females) and Icelandic-Canadians	Plasma PL	Young Icelandic-Canadians had lower levels of EPA than the middle and oldest age groups		1.8 fold increase in oldest group compared to the youngest	

	(60 males and 59 females), 20-69 years					
[143]	54 women, 43-60 years: 19 premenopausal (~48 y), 16 postmenopausal not receiving HRT (~52 y) and 19 postmenopausal receiving HRT (~51 y)	Serum PL	DHA levels were significantly lower in premenopausal women than postmenopausal women. Those receiving HRT had significantly lower levels of DPA.			1.3 fold increase in postmenopausal women without HRT vs premenopausal
[144]	338 women; alcohol intake: abstainers (n=254, ~24,2 y), occasional (n=45, ~27,9 y) and habitual (n=8, ~30,5 y)	Plasma and RBC	DHA and AA correlates positively with maternal age			↑ in plasma (µg/ml et %) ↑ in RBC (%)
[49]	99 Icelandic women, 18 to 73 y (~45.8 y)	RBC	Proportions of total n-3 PUFA, EPA, and DHA correlated positively with age		↑	↑
[145]	200 Japaneses, 126 males and 74 females, ~50 y (<35 to ≥65 y)	Serum and RBC total lipids	EPA, DHA, n-3: n-6 ratio and EPA: AA ratio increased with age (stronger effect in serum):		Group ≥65 y compared to group <35 y: 2.3 fold increase in serum and 2 fold increase in RBC	Group ≥65 y compared to group <35 y: 1.7 fold increase in serum and 1,2 fold increase in RBC

AA: arachidonic acid, EPA: eicosapentaenoic acid: DHA, docosahexaenoic acid: DPA: docosapentaenoic acid, AT: adipose tissue, PUFA: polyunsaturated fatty acids, FA, fatty acids, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PS: phosphatidylserine, CE: cholesteryl esters, NEFA: non-esterified fatty acids, RBC: red blood cells, HRT: Hormone receiving therapy, BMI: body mass index,

799 Table 2: Blood fatty acid modulation by age after an omega-3 fatty acid supplementation

Reference	***n*, sex and age**	**Blood pool**	**Omega-3 supplementation**	**Age effects**
[59]	n=115, 60 men and 55 women, 20 to 45 years	RBC	5 doses (0, 300, 600, 900, 1800 mg) of EPA+DHA (fish oil) for ~5 months	Lower Omega-3 Index (O3I) status (P<0.0001) and older age (P=0.02) each predicted greater increases in O3I with supplementation
[62]	24–28 participants in each age group (except as noted in the tables), young adult = 18-34 y (~23 y) and elderly group = ≥65 y (~74 y)	Plasma	Two supplementations: n-3 supplement enriched in DHA (680 mg DHA/d plus 323 mg EPA/d) for 3 weeks, or a supplement enriched in EPA (1480 mg EPA/d plus 250 mg DHA/d) for 6 weeks	Expressed as % of total fatty acids: At baseline, total n-3 PUFA, EPA and DPA higher in elderly (32%, 100% and 25% respectively); Expressed as concentration (mg/L): At baseline, total n-3 PUFA, 18:3n-3, DHA, DPA and EPA higher in elderly (74%, 40%, 63%, 85% and 142% respectively); After supplementation: no higher effect with increasing age
[63]	15 young (22-35 y) and 10 older (51-71 y) women	Plasma	Daily 1680 mg EPA and 720 mg DHA for 3 months	Older women had a significantly higher increase in EPA and DHA than did young women (EPA: 10-fold vs 8-fold and DHA: 2.5-fold vs 2-fold)
[64]	6 young (23-33 y) and 6 older (51-68 y) women	Plasma	Daily 1680 mg EPA and 720 mg DHA for 3 months	At baseline there was no difference in percentage of EPA and DHA between young and older women; however, after 3 mo of (n-3) fatty acid supplementation, older women had a significantly higher percentage of EPA and DHA: EPA: 10-fold vs 5-fold and DHA: 2.5-fold vs 1.6-fold
[65]	10 young (5 men and 5 women, ~22 y) and 10 elderly (5 men and 5 women, ~ 75 y)	Plasma	EPA-enriched supplement (1.4 g/d of EPA and 0.2 g/d of DHA) for 6 wk	Before and after the EPA supplement, fasting plasma EPA was higher in the elderly (by 85% and 67% respectively)

[66]	Young (18-42 y; n=93) and old (53-70 y; n=62) men	Plasma and MNC PL	Placebo (corn oil) or 1.35, 2.7, or 4.05 g EPA/day for 12 wks	In both plasma and MNC PL : at baseline, EPA and DPA increase with age while after supplementation, only EPA increases in old men; at baseline, EPA, DPA and DHA respectively ~1.3, ~1.1 and ~1.4 higher in older in plasma and EPA and DHA respectively ~1.3 and ~1.25 higher in older in MNC; with High-EPA supplementation: EPA and DPA respectively ~1.6 and ~1.3 higher in plasma and EPA ~1.4 higher in MNC
[61]	Elderly (n=9, 5 males and 4 females, 74 y) and young (n=10, 5 males and 5 females, 24 y)	Plasma	680 mg/day of DHA and 320 mg/day of EPA for 3 weeks, followed by 2 weeks of wash-out	Higher baseline plasma EPA in elderly group; In response to the supplement, plasma DHA rose 42% more in the elderly but EPA responded similarly in both groups
[67]	n=193 (101 women, 92 men), 20–79 y	Plasma PC, CE, NEFA and TG; MNC; RBC; PLAT; BU; AT	EPA+DHA equivalent to 0, 1, 2 or 4 portions of oily fish per week, for 12 months	At baseline, EPA in AT and DHA in plasma TG and AT higher with increasing age; Following supplementation, EPA in plasma TAG higher with increasing age while DHA in AT smaller with increasing age
[60]	92 Danish women: half premenopaused (~43 y) and half postmenopaused (~56 y), 18-70 y	PLAT, AT	2,2 g of marine n-3 PUFA (38,5% EPA, 25,9% DHA and 6,0% DPA) or control oil (thistle oil) daily for 12 weeks	Baseline contents of EPA, DPA and DHA were all significantly lower ($P<0.05$) in premenopausal group both in platelets and adipose tissue, except for EPA in platelets ($P=0.05$); After supplementation, increase in platelets and adipose tissue was, however, the same in both groups

EPA: eicosapenaenoic acid, DHA, docosahexaenoic acid, DPA: docosapentaenoic acid, PLAT: platelets, AT: adipose tissue, PUFA: polyunsaturated fatty acids, PC: phosphatidylcholine, CE: cholesteryl esters, NEFA: non-esterified fatty acids, MNC: mononuclear cells, RBC: red blood cells, BU, buccal cells,